
Tuesday, June 10

59-52

Session TA4
Room 4
8:30 - 11:30 a.m.

**Effect of Microgravity on Bone
Tissue and Calcium Metabolism**

HUMAN BONE TISSUE CHANGES AFTER LONG-TERM SPACE FLIGHT: PHENOMENOLOGY AND POSSIBLE MECHANICS

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INTRODUCTION:

Bone loss occurs when terrestrial vertebrates adapted to earth gravity are exposed to microgravity. We quantified the bone loss on Russian Cosmonauts after long term space flights. Bone loss could limit duration of space missions in the future. We report here the results obtained from pre and postflight cosmonaut bone mineral density determinations since 1990. Aims are: a) to define bone loss (anatomic location of loss, magnitude of loss) and b) to offer possible mechanisms of bone tissue changes that could be used in countermeasure development.

OBJECTS AND METHODS:

Twenty one cosmonauts were studied before and after long-term space flight (4.5-14.5 months) using dual energy X-ray absorptiometry (DEXA) on a Hologic QDR 1000 housed in Star City. Bone mineral content (BMC, g) and bone mineral density (BMD, g/cm²) were measured with regional analysis of the whole body scan (pencil beam mode) and with local analysis of the hip scan and the lumbar spine scan.

RESULTS:

The direction and magnitude of bone changes reveal a distinct dependence on the position of the skeletal segment along the gravity vector and confirms previously described tendencies. BMD in the lower half of the skeleton is decreased. The mean BMD decrease for all cosmonauts (% per month) was: lumbar spine -1%, proximal femur -1.3%, pelvis -2%. In the upper body (head, ribs, arms) a tendency toward an increase of BMC was sometimes indicated. A good correlation between the amount of bone loss in different parts of the skeleton and their loading by body weight in 1G was observed. The second important peculiarity was the extremely high interindividual differences. The high individual variations of changes after flight compared with preflight was established: in lumbar spine (BMD) from +3% to -12%; in pelvis (BMC) from -1% to -22%; in the femoral neck (BMD) from 0% to -17%. We were unable to correlate the magnitude of changes with flight duration alone. Bone changes have shown a slight negative correlation with age of cosmonauts and volume of on board physical exercises.

CONCLUSION:

The data is comparable to results of human head down bed rest studies and animal and bone culture in vitro experiments under actual and simulated microgravity. The bone loss in the lower part of the skeleton can be described as local osteopenia, resulting from the selective inhibition of the physiologic bone tissue remodeling in the weight bearing parts of the skeleton and is a result of the adaptive response to the decrease in mechanical load (deformation). There are reasons to presume the local osteopenia is the manifestation of the tissue adaptation resulting from changes in local factors regulating bone metabolism. The variability of the response of the human skeleton is associated in part with individual differences in peak bone mass peak, which has a genetic component. The regional increase in the mineral content may be a secondary response to other physiologic factors such as body fluid redistribution in the cranial direction in microgravity and/or hormonal and biochemical changes occurring in space flight.

PREDICTION OF FEMORAL NECK BONE MINERAL DENSITY CHANGE IN SPACE

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INTRODUCTION

Bone mineral density (BMD) losses in the femoral neck have been reported to average 1.16% of the preflight BMD with a standard deviation of $\pm 0.85\%$ for each month aboard the MIR space station. Actual changes in BMD have ranged from a loss of 0.189 gm/cm^2 (2.39% per month) to a gain of 0.034 gm/cm^2 , (0.57% per month). All cosmonauts took part in an exercise program using a treadmill, bicycle ergometer and bungee cords. There has been no explanation for the large variation in the rate of BMD loss among individuals, but it has been speculated that the degree of individual compliance with the exercise program as well as individual exercise habits prior to flight are contributors. We now find that historical BMD changes in the femoral neck during spaceflight could have been fairly well predicted for men by a combination of factors related to biomechanical strength of the femoral neck, body composition and flight time.

METHODS

Using data from 15 male subjects that flew on MIR prior to 1995 with flight durations ranging from 117 to 438 days, pre-flight values of the femoral neck BMD ("B"), bone length ("L") in cm. and width ("W") in cm., along with average lean leg mass "M" (g), height ("H") in cm., were incorporated into a regression model predicting change in femoral neck BMD. Two terms involving functions of these measurements were used along with the flight time ("t") in days in the following prediction model, which produced the best fit:

$$\Delta BMD = C_0 + C_1 t^{1/2} + C_2 \frac{M}{H} t^{1/2} + C_3 BLW^2 t^{1/2}$$

where the ΔBMD is the change in BMD and the C_k are constants. Hip DEXA scans were used to obtain BMD, length and width measurements (see Figure 1). Average lean leg mass was obtained from whole body DEXA scans (Hologic QDR-1000).

RESULTS

The model explained 85.6% of the variation in BMD change over individuals, with residuals ranging from -0.052 to $+0.052 \text{ gm/cm}^2$ and an RMS error of 0.029 gm/cm^2 (see Figure 1). Taking into account the uncertainty in the estimated parameters, femoral neck BMD change for a new individual could be predicted by this model with a standard error of prediction ranging from 0.033 to 0.039 gm/cm^2 . By contrast the simple linear model based only on time, explains only 29.3% of the variation and has an RMS error of 0.056 gm/cm^2 with residuals ranging from -0.077 to $+0.110 \text{ gm/cm}^2$.

CONCLUSION

The prediction model for the femoral neck BMD may be used to anticipate serious bone loss for some space station users so that appropriate countermeasures can be implemented. Because the model is empirical, it requires validation by applying it to subjects whose BMD data was not used in the fitting process. This validation is being conducted using new pre and post flight DEXA measurements as the data become available. To date, the model predicted the change in BMD to within 0.002 gm/cm^2 for one new MIR subject and even predicted the change to within 0.024 and 0.066 gm/cm^2 for two bedrest subjects without exercise.

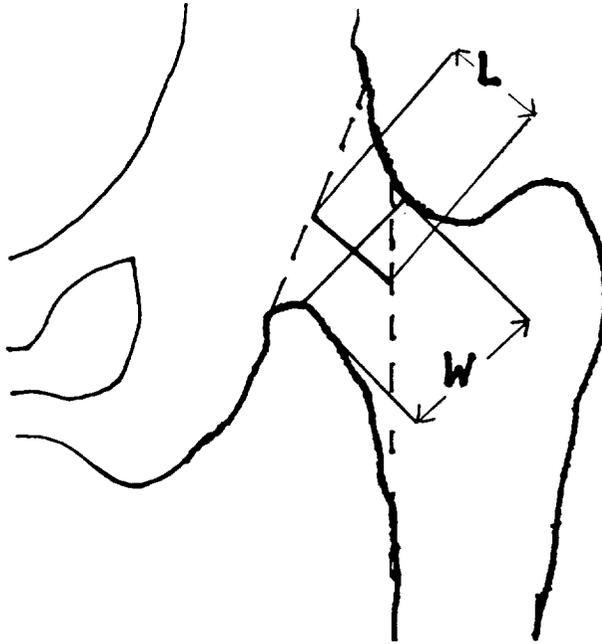


Figure 1. Hip neck measurements

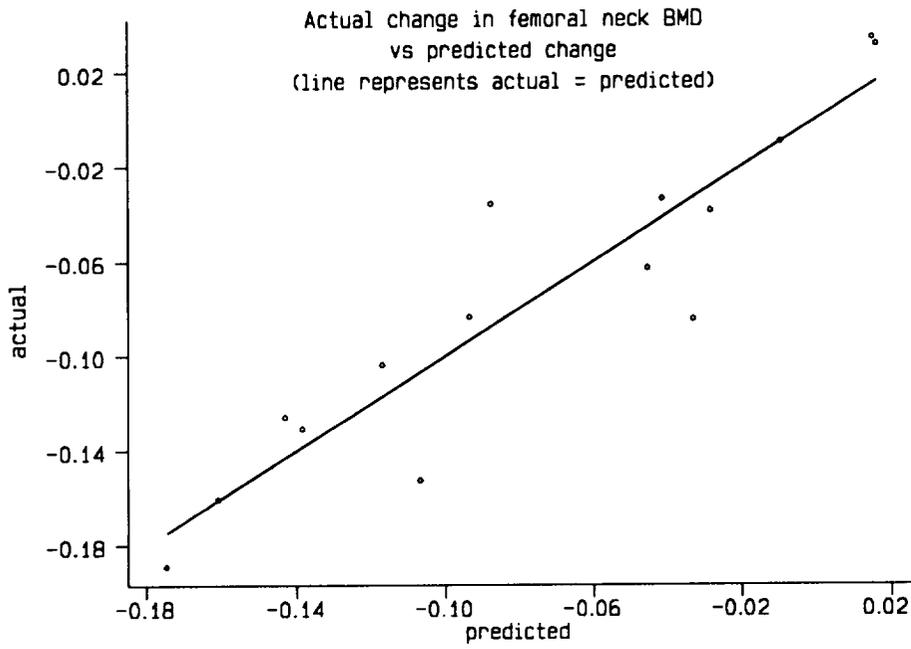


Figure 2. BMD change: actual vs. predicted

DIETARY CALCIUM IN SPACE

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INTRODUCTION

Deficits in calcium intake are associated with increased blood pressure, decreased bone mineralization and impaired calcium metabolism. Calcium losses due to exposure to microgravity may result in a similar constellation of outcomes.

METHODS

To test that hypothesis, fourteen 7-week-old, male spontaneously hypertensive rats (SHR) were flown on STS-80, an 18 day shuttle mission. Beginning at 3 weeks of age, half the rats were fed a low calcium diet (0.2%) and half were fed a high calcium (2.0%) diet. The animals were maintained on the diets throughout the experiment.

RESULTS

Preliminary results indicate that systolic blood pressure, measured in conscious SHR 3 hours after landing using an indirect tail cuff method, was somewhat lower in the flight animals relative to concurrent ground controls ($p=0.053$). When anesthetized with halothane (2% in O_2) just prior to catheterization for blood sampling, direct arterial blood pressure was found to be significantly higher ($p<0.0001$) in the flight animals than the control animals in both diet groups (+18 mmHg on average). The differences in blood pressure may have been related to variations in vascular smooth muscle function. Mesenteric resistance vessels from flight animals had smaller maximal contractions to norepinephrine than control animals ($p<0.0001$) and showed poorer relaxation to acetylcholine, calcium and sodium nitroprusside ($p<0.0001$). Ionized calcium values between diet groups were much closer together in the flight animals (1.34 vs 1.38 mmol/L, $p<0.01$) than the controls (1.24 vs 1.36 mmol/L, $p<0.0001$). Parathyroid hormone values for flight animals were 198 vs 127 pg/ml for the low and high calcium groups respectively ($p<0.05$). Vivarium control values were 145 vs 46 pg/ml ($p<0.001$). These values indicate that microgravity increased PTH levels while preserving the dietary difference. Basal free intracellular calcium values were decreased in platelets from flight animals ($p<0.05$) while thrombin and ionomycin stimulated calcium levels did not differ from control animals. Finally, animals on low calcium diets were reported by the crew to be more active on orbit. Ground observations confirmed their report and found that the low calcium vivarium controls were more active as well.

CONCLUSIONS

Overall, the preliminary data indicate that exposure to microgravity had a profound effect on blood pressure regulation, vascular function, calcium metabolism and activity. As we continue our assays and analysis, additional observations and insights will be forthcoming.

CALCIUM METABOLISM DURING EXTENDED-DURATION SPACE FLIGHT

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The effect of near-weightlessness on the human skeletal system is one of the most critical concerns in safely extending space missions. Minerals are lost from bones during flight as a result of skeletal unloading, thereby increasing calcium excretion in the urine. Bone loss contributes to the increased risk of renal-stone formation associated with high concentrations of solutes in the urine during and after flight. This study was designed to examine calcium and bone homeostasis during a 115-day mission on board the Mir space station.

Three male subjects participated, and data were collected before, during and after the mission. Blood and urine samples were collected, aliquoted, and frozen until postflight analysis, except for blood ionized calcium and pH, which were determined in "real-time" with a portable clinical blood analyzer. Biochemical and endocrine indices of bone metabolism were determined. Calcium absorption and kinetics were studied using a dual isotope technique. Oral (^{43}Ca , 125 μg) and intravenous (^{46}Ca , 8 μg) tracers were administered (oral tracer followed 1 hr later with I.V. tracer), with timed blood, urine, and saliva samples being collected over 3-5 days. Fecal samples were also collected during pre- and postflight sessions. Calcium isotopic enrichments in biological samples were determined using thermal ionization mass spectrometry. Absorption data were determined using the ratio of the two isotopes in samples obtained > 24 hours post dosing.

Serum concentrations of intact parathyroid hormone were reduced almost 30% during flight compared to preflight. Serum 25-OH Vitamin D (calcidiol) decreased during winter training in Russia (compared to autumn in Houston), and decreased further during flight. Serum 1,25(OH)₂ Vitamin D (calcitriol) concentrations were also reduced almost 30% during flight. Serum osteocalcin tended to decrease during winter, and was increased or unchanged during flight. Bone-specific alkaline phosphatase concentrations were an average of 39% lower on flight day 14 and 11% lower on flight day 110 than preflight values. Serum total calcium was unchanged, the ionized fraction increased slightly, and venous whole blood pH did not change. Urinary calcium excretion was higher during flight than before. Urinary collagen metabolite excretion (n-telopeptide and pyridinium crosslinks) was almost 40% greater than the preflight level. Calcium absorption decreased during ($38 \pm 18\%$ below preflight values on flight day 110) and after flight ($56 \pm 9\%$ below preflight on landing day, $n = 2$; and $29 \pm 28\%$ below preflight on return +6 days). By 3 months postflight, calcium absorption had returned to $4 \pm 20\%$ below preflight values. These data showed that inflight bone loss is associated with increased resorption and decreased formation. Calcium balance is further altered by decreased PTH activation of calcidiol, with subsequent decreased calcium absorption. These data will be critical for assessing the efficacy of countermeasures to the weightlessness-induced bone loss. Further studies are needed to develop techniques to maintain bone and calcium homeostasis during extended-duration space flight.

EXTERNAL IMPACT LOADS ON THE LOWER EXTREMITY DURING JUMPING IN SIMULATED MICROGRAVITY AND THE RELATIONSHIP TO INTERNAL BONE STRAIN

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INTRODUCTION

Exercise protocols have been developed to counteract the adverse reactions to space travel; however, to date no modality has been adequate in preventing space-flight induced osteoporosis. Exercises which afford functional weight bearing and more importantly deliver high dynamic loads to the lower extremities are thought to stimulate bone deposition by providing strain and strain rates to maintain bone integrity (Cavanagh et al., 1992). In support of this hypothesis, experimental results from animal studies have shown that strains above 0.1% are associated with substantial bone formation (Rubin and Lanyon, 1985). The relationship between the external impact forces and the internal bone strains, however, has yet to be elucidated. The purpose of this investigation, therefore, is to predict the strains found in simulated microgravity during jumping exercises which are known to impart high impact forces to the body in the earth's gravitational field.

METHODS

A zero gravity simulator (ZGS) was constructed using ten foot long latex cord and rope to suspend subjects from the ceiling in a supine position (Figure 1). The cords were attached to the center of gravity of the lower extremity segments. Body segment masses were calculated using regression equations and anthropometric measurements to assure that the tension in the cord matched the weight of each segment (Vaughan et al., 1992). Rope also supported the subject at chest and waist harnesses. A gravity replacement system consisting of two steel springs, attached at the waist in front and back, was used to tether the subject to the wall. The springs were tensioned in order to provide a force equal to 30-100% of the subject's body weight. Six subjects (four females and two males, ages 24-35) performed a countermovement jump in the zero gravity simulator with their feet landing on a wall mounted force plate.

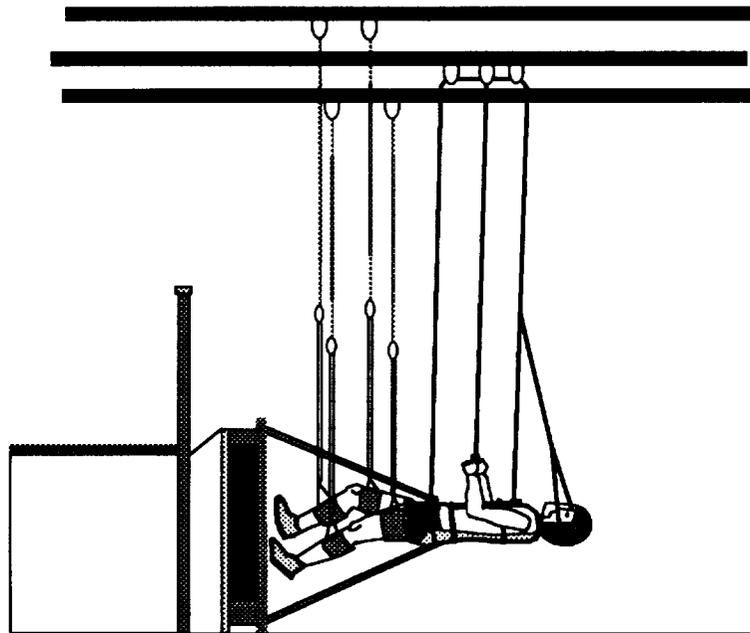


Figure 1: Schematic of Subject Suspended in Zero Gravity Simulator

In order to predict the internal bone strains achieved during jumping, data from drop tests of three cadaveric lower limbs were used. Tibial strains were measured by implanting two parallel K-wires into the distal, anteromedial tibia and clamping an extensometer on the exposed wires. The potted specimens were then secured in a custom drop test apparatus which enabled the peak load and impact velocity to be varied by altering the additional weight and drop height. Tibial strains and force profiles were recorded for each drop.

RESULTS

Peak forces during landing in the ZGS were determined and an average was calculated for trials at the same tension level in the springs for each subject. Peak forces ranged from 1400 N at the lower spring tensions to 3100 N at the higher tensions, corresponding to 1.7 - 4.0 times the subject's body weight. Results from the cadaveric limbs during the drop test showed peak loads ranging from 300-2300 N (0.5-2.5 time body weight) and tibial strains between 0.02 and 0.1%. Correlation between the peak load and the tibial strains resulted in a linear correlation coefficient, r , of 0.61 and the regression equation:

$$\text{Tibial Strain (\%)} = 0.0000245 * \text{Peak Load (N)} + 0.00566 \quad (p < 0.001) \quad (1)$$

Assuming that each foot was loaded symmetrically on the force plate during the jumping trials, the peak load during landing in the ZGS was halved and the predicted tibial strain for each foot was calculated according to Equation 1. This resulted in tibial strain in the ZGS that ranged from 0.0083 to 0.032%.

DISCUSSION

This investigation reveals the first attempts to relate external ground reaction force to the resulting internal bone strains in order to develop an effective countermeasure to space-flight induced osteoporosis. The peak loads in the zero gravity simulator during jumping are lower than values reported in the literature for 1G (McNitt-Gray, 1993). The results do, however, compare well to previous studies of jumping in simulated microgravity. Vrijotte (1991) reported average peak load values of 2043 N (± 1097) and 2055 N (± 922) with spring tension at 50% body weight. At similar conditions, the current investigation found peak loads between 1360 and 2268 N. The predicted peak tibial strains in the ZGS fell short of the known magnitude of 0.1% strain necessary for maintaining the integrity of bone (Rubin and Lanyon, 1985). Nonetheless, there were statistically significant relationships between peak load and strain in the cadaveric data suggesting that subjects may need to modify their exercises by altering their landing or by utilizing added mass. Furthermore, two thirds of the subjects could elicit loads under the calcaneus which is important given the fact that there is an absence of heel loading in exercises currently used during extended orbital missions.

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BONE LOSS DURING LONG TERM SPACE FLIGHT IS PREVENTED BY THE APPLICATION OF AN SHORT TERM IMPULSIVE MECHANICAL STIMULUS

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INTRODUCTION

Bone mass is regulated by physical activity, and significant changes in bone mass have been observed when physical activity has been reduced or increased^(1,2). Lanyon *et al*⁽³⁾ have hypothesised that the skeleton will regulate its mass in response to changes in activity, so that an increase in activity will trigger bone formation while a decrease in activity will have the opposite effect. In long term space flight, where the normal gravitational field is absent, the mechanical forces on the skeleton are reduced and are changed in character. This reduced skeletal loading results in a reduction in bone mass. Currently used exercise techniques can maintain muscle mass but the stimulus provided by this exercise does not prevent loss of bone mass particularly from bones of the lower limb. By applying an impulsive load (to mimic the heel strike transient) to the lower limb of an cosmonaut during a long term space flight (5 months), this study tests the hypothesis that the bone cells can be activated by an appropriate external mechanical stimulus to maintain bone mass throughout prolonged periods of weightlessness.

METHODS

An impulsive loading device was developed to mimic the stimulus provided by the heel strike transient which occurs during normal locomotion. The device comprised of two heel plates supported by two spring loaded actuators, each with identical spring characteristics. The springs were compressed by the cosmonaut loading the heel plates; at the end of travel of the 'test' actuator a 1 kg mass was released which impacted on the left heel plate. This produced an impact spike similar to that experienced at heel strike during a brisk walk. This impulsive loading stimulus was applied by the cosmonaut at a rate of approximately 0.5 Hz on a daily basis for 500 cycles. Pre- and post-flight measurements of bone mineral density (BMD) were made using a DXA (Dual Energy X-ray Absorptiometry) scan on both ossa-calcis. To minimise variation in position between successive scans, the ossa-calcis and lower limb were supported in a specially constructed jig during scanning. Follow-up scans were made at 1, 3, 5 and 9 weeks post-flight. Additional scans were performed on both femoral necks at these same time points to determine the effect of the impulsive loading at a skeletal site remote from the point of application of the applied stimulus.

RESULTS

The results obtained from the ossa-calcis (Figure 1) demonstrate that BMD is maintained throughout the period of the space flight on the ossa-calcis which received the mechanical stimulus, while it is reduced by up to 7% on the ossa-calcis which received no stimulus. Post-flight, changes in BMD in both the stimulated and non-stimulated ossa-calcis are suggestive of a process of equalisation of BMD in the ossa-calcis. For the femoral neck, no positive effect of the mechanical stimulus was noted (Figure 2).

CONCLUSION

This work suggests that high frequency transient loading with consequent high strain rates are important in maintaining peripheral bone mass.

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Region of Interest on Os-Calcis

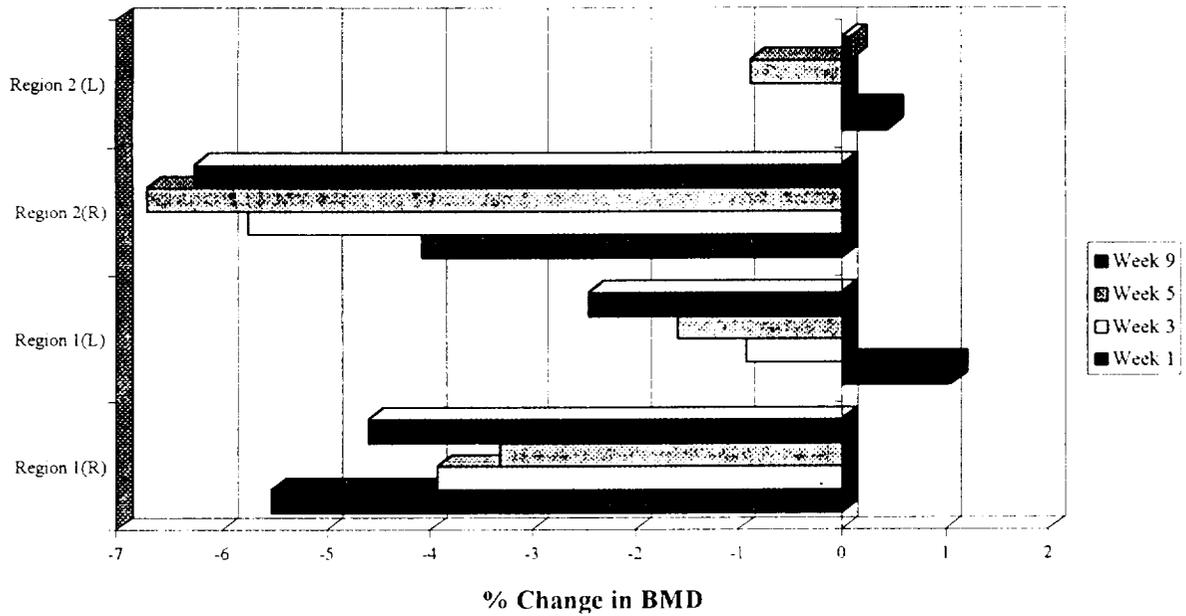


Figure 1. Percentage change in os-calcis BMD relative to pre-flight values at increasing times post-flight. The left os-calcis received the mechanical stimulus.

Region of Femoral Neck

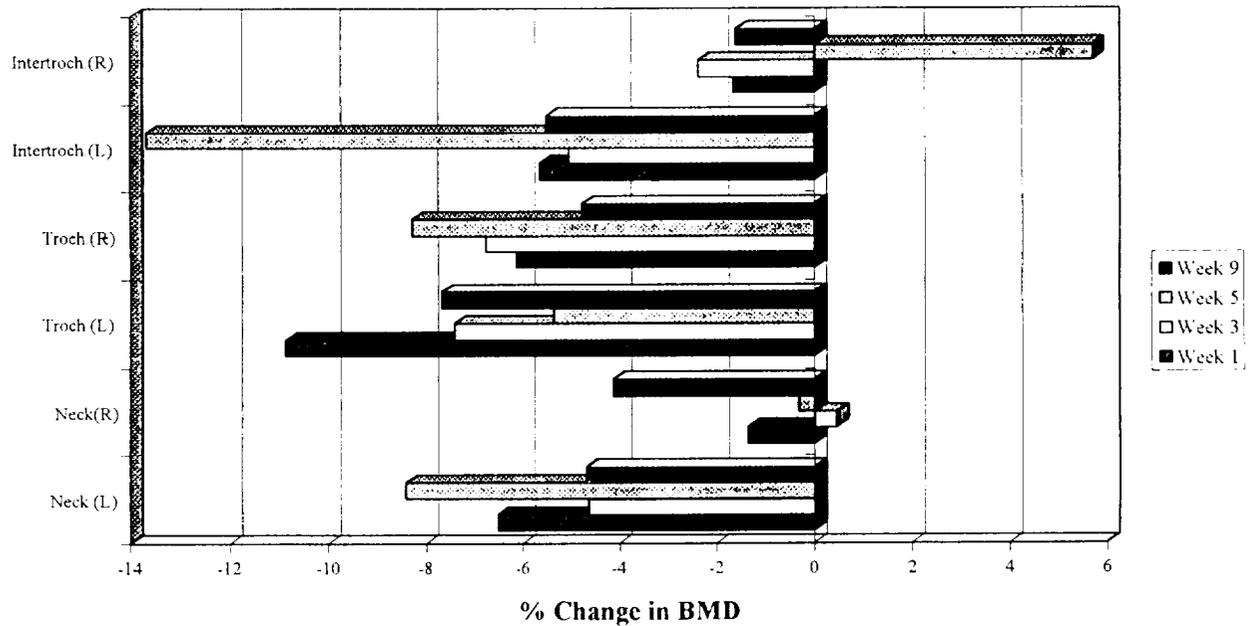


Figure 2. Percentage change in femoral neck BMD relative to pre-flight values at increasing times post flight. The mechanical stimulation was applied to the left side.